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CIII.—*Dibenzoylglucoxylose: A Natural Benzoyl Derivative of a New Disaccharide.*

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In a previous communication (this vol., p. 767) it was shown that the bitter taste which characterises the leaves and stems of the Australian shrub *Daviesia latifolia*, R. Br., is due to the presence of a considerable proportion of a crystalline substance, *dibenzoylglucoxylose*, which melts at 147—148°, and possesses the empirical formula $C_{25}H_{28}O_{12},H_2O$. This substance appears to represent an entirely new type of naturally occurring compounds, and, since a comparatively large amount of it was available, the opportunity has been utilised of subjecting it to a complete examination.

Properties of Dibenzoylglucoxylose.

Dibenzoylglucoxylose is only sparingly soluble in cold water, ether, or chloroform, but readily so in alcohol or hot water. In the nearly pure condition it is best crystallised from the latter solvent, when it separates in well-formed prismatic needles, containing one molecule of water of crystallisation. It possesses an extremely bitter taste, and as this property is also exhibited, although in a less degree, by penta-acetyldextrose (*Ber.*, 1889, **22**, 1466), it is possibly common to the acyl derivatives of the sugars. The substance has no reducing action on Fehling's solution. It is strongly lævorotatory, its specific rotation having been determined with the following result:

0·5094,* made up to 20 c.c. with methyl alcohol, gave $\alpha_D - 5°26'$ in a 2-dcm. tube, whence $[\alpha]_D - 106·7°$.

Penta-acetyldibenzoylglucoxylose, $C_{11}H_{13}O_{10}(CO{\cdot}C_6H_5)_2{\cdot}(CO{\cdot}CH_3)_5$.

This compound was prepared primarily for the purpose of obtaining a derivative of dibenzoylglucoxylose which would be suitable for the determination of the molecular weight of the latter by the cryoscopic method. A quantity of dibenzoylglucoxylose was heated for several hours with an excess of acetic anhydride, and the product then poured into water, when the *acetyl* derivative quickly separated in the solid state. This was collected and purified by crystallisation from absolute acetic acid, from which it was deposited in slender needles, melting at 203°. The substance was analysed, and its molecular weight determined, with the following results:

0·1002 gave 0·2094 CO_2 and 0·0473 H_2O. C = 57·0; H = 5·2.
0·2964 in 26·3 nitrobenzene gave $\Delta t - 0·110°$. M.W. = 717.
0·2609 ,, 27·4 absolute acetic acid gave $\Delta t - 0·048°$. M.W. = 778.
$C_{35}H_{38}O_{17}$ requires C = 57·5; H = 5·2 per cent. M.W. = 730.

The number of acetyl groups in this compound cannot be ascertained from its analysis, but an investigation of the action of alkali hydroxides on it and the original dibenzoyl compound, as described below, indicated that five acetyl groups were present. It is, however, evident from the above results that the original compound, dibenzoylglucoxylose, is correctly represented by the formula $C_{25}H_{28}O_{12}$, thus confirming the previous determination of its molecular weight (*loc. cit.*) by the microscopic method.

Penta-acetyldibenzoylglucoxylose is sparingly soluble in alcohol, but more readily so in benzene, chloroform, or acetic acid. It is quite insoluble in water, and possesses only a faintly bitter taste.

Alkaline Hydrolysis of Dibenzoylglucoxylose.

Formation of Glucoxylose, $C_{11}H_{20}O_{10}$, *and Benzoic Acid.*

In order to ascertain the best conditions for the alkaline hydrolysis of the compound, a number of preliminary experiments were first conducted with the use of a known volume of standard alkali under varying conditions, and determining the amount of alkali

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[*] Anhydrous substance.

consumed by subsequent titration with acid in the presence of phenolphthalein. It was thus found that concordant results could not be obtained when the substance was heated with aqueous or alcoholic alkali, since the amount of the latter neutralised in the reaction was proportional to the time of heating. Very satisfactory results were obtained, however, by the use of alkali hydroxides at the ordinary temperature. In a series of experiments in which the substance was dissolved in aqueous sodium hydroxide, and the mixture kept for lengths of time varying from five minutes to twenty-four hours, the same amount of alkali was required for the hydrolysis in each case. For periods of less duration than five minutes the hydrolysis was incomplete. The alkali liquids resulting from the above treatment yielded benzoic acid on acidification, and it was thus evident that with the use of cold alkali the benzoyl groups were rapidly eliminated from the compound. By employing this method for the determination of the benzoyl groups, the following results were obtained:

0·1328 * required for hydrolysis 5·1 c.c. *N*/10-NaOH. $C_6H_5\cdot CO = 40\cdot 3$.

0·2572 * required for hydrolysis 9·9 c.c. *N*/10-NaOH. $C_6H_5\cdot CO = 40\cdot 4$.

0·1605 * required for hydrolysis 6·1 c.c. *N*/10-NaOH. $C_6H_5\cdot CO = 39\cdot 9$.

$C_{11}H_{18}O_{10}(CO\cdot C_6H_5)_2$ requires $C_6H_5\cdot CO = 40\cdot 4$ per cent.

A similar quantitative experiment was conducted with the pentaacetyl derivative of the above compound for the purpose of determining the number of acetyl groups present. In this case, however, owing to the sparing solubility of the substance, it was necessary to keep the mixture of finely powdered material and aqueous sodium hydroxide for several days at the ordinary temperature before complete solution was effected. The result then obtained was as follows:

0·1050 * required for hydrolysis 9·85 c.c. *N*/10-NaOH.
Calculated amount required for
$C_{11}H_{13}O_{10}(CO\cdot C_6H_5)_2(CO\cdot CH_3)_5 = 10\cdot 1$ c.c.

It is thus evident that the acetyl derivative of the compound contains five acetyl groups, in addition to the two benzoyl groups.

Having ascertained by experiments on the small scale that the benzoyl groups of dibenzoylglucoxylose can readily be eliminated as above described, it was next deemed desirable to subject larger quantities of material to the same treatment in order to isolate and examine the hydrolytic products. Accordingly, 25 grams of dibenzoylglucoxylose were dissolved in hot water, and the solution rapidly cooled, after which 600 c.c. of $N/3$-aqueous barium hydroxide were added, and the mixture kept for several hours at the ordinary temperature. The barium was then exactly precipitated by the addition of an equivalent amount of dilute sulphuric acid, and the mixture filtered. The precipitate of barium sulphate was washed with ether, and the filtrate also thoroughly extracted with the same solvent, the ethereal liquids being then united, dried, and the solvent removed. A crystalline residue (12 grams) was thus obtained, which separated from hot water in colourless leaflets, melting at 121°. This substance was identified as benzoic acid. (Found, $C=68{\cdot}6$; $H=5{\cdot}0$. Calc., $C=68{\cdot}9$; $H=4{\cdot}9$ per cent.)

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* Anhydrous substance.

The aqueous liquid which had been extracted with ether for the removal of the benzoic acid was concentrated under diminished pressure at 70° to a small bulk. A syrupy residue was thus obtained, which did not crystallise after keeping for several days. In order to remove the last traces of water from this material, it was dissolved in hot methyl alcohol, and the solution evaporated under diminished pressure, the process being ultimately repeated in a vacuum desiccator. The material was then obtained as a nearly colourless, amorphous, horn-like solid, which rapidly absorbed moisture on exposure to the air. Many attempts were made to obtain the substance in a crystalline state, but hitherto without success. An analysis of the amorphous substance gave the following result:

0·1086 * gave 0·1674 CO_2 and 0·0622 H_2O. $C=42{\cdot}0$; $H=6{\cdot}4$.

$C_{11}H_{20}O_{10}$ requires $C=42{\cdot}3$; $H=6{\cdot}4$ per cent.

The above-described substance is thus seen to possess the formula $C_{11}H_{20}O_{10}$, and the hydrolysis of the original compound may therefore be represented by the following equation:

$$C_{25}H_{28}O_{12} + Ba(OH)_2 = Ba(C_7H_5O_2)_2 + C_{11}H_{20}O_{10}.$$

A consideration of the properties of the substance $C_{11}H_{20}O_{10}$, as described below, renders it evident that it is a disaccharide which contains both a dextrose and xylose complex, and hence it has been designated *glucoxylose.*

Properties of Glucoxylose, $C_{11}H_{20}O_{10}$.

Glucoxylose is extremely soluble in water, readily so in methyl alcohol, but only moderately so in absolute ethyl alcohol. Its aqueous solutions have a sweet taste, and are lævorotatory, but do not reduce Fehling's solution, and do not yield an osazone.

* Dried at 130°.

The optical rotation of glucoxylose could not be determined directly on account of its hygroscopic nature and the deepening of colour produced on drying it at an elevated temperature. The determination of its rotation was therefore accomplished indirectly in the following manner:

1·1050 Grams* of dibenzoylglucoxylose (=0·6630 gram of glucoxylose) were hydrolysed by cold aqueous barium hydroxide, and the barium was then exactly precipitated by its equivalent of sulphuric acid. The precipitate of barium sulphate was subsequently removed by filtration, washed well with water, and the filtrate and washings made up to the volume of 100 c.c. This solution, containing the glycoxylose, together with some benzoic acid, gave $\alpha_D - 0°29'$ in a 2-dcm. tube, whence $[\alpha]_D - 36·5°$. A second determination, performed in a similar manner, gave $[\alpha]_D - 35·8°$. It may be considered that the accuracy of these results would not be appreciably influenced by the small amount of benzoic acid present in the solution.

A quantity of glucoxylose was heated for some time with acetic anhydride for the purpose of preparing an acetyl derivative, but the product could not be obtained in a crystalline state, and therefore was not further investigated.

Acid Hydrolysis of Glucoxylose.

Formation of Dextrose and Xylose.

To an aqueous solution of glucoxylose, prepared from 20 grams of the dibenzoyl derivative, such an amount of sulphuric acid was added as to have the latter represent 5 per cent. of the total liquid, and the mixture then heated at 100° for about an hour. The sulphuric acid was afterwards removed by the addition of an exact equivalent of barium hydroxide, and the mixture filtered. The filtrate, which was now dextrorotatory, was concentrated under diminished pressure until the water was removed as completely as possible. The residual syrup was then dissolved in hot absolute alcohol, and ethyl acetate cautiously added to the solution, when, on cooling, a syrupy deposit was produced, from which the mother liquor was decanted. The latter was kept for several days, when hard, wart-like aggregates of anhydrous crystals, melting at 146°, were gradually formed. This crystalline substance was collected and analysed. (Found, C=40·0; H=6·7. $C_6H_{12}O_6$ requires C=40·0; H=6·7 per cent.)

An aqueous solution of the substance exhibited the phenomenon of mutarotation, and, after equilibrium had been established, its specific rotatory power was determined:

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* Anhydrous substance.

0·1180, made up to 20 c.c. with water, gave $\alpha_D + 0°37'$ in a 2-dcm. tube, whence $[\alpha]_D + 52·3°$.

The substance yielded an osazone, which melted and decomposed at 210°.

These results thus conclusively prove that dextrose is one of the hydrolytic products of the disaccharide, designated as glucoxylose.

The above-mentioned first deposit of syrupy liquid yielded a mixture of osazones melting at 185°, and it was therefore evident that it contained some other sugar besides dextrose. The results obtained by the analysis of the original crystalline compound, and the primary products of its hydrolysis, had furthermore rendered it highly probably that this other sugar was a pentose. In order to effect its isolation, the whole of the sugar remaining from the above acid hydrolysis was dissolved in 300 c.c. of water, a small amount of yeast added, and the mixture kept at a temperature of 35—40° for several days. After the fermentation had ceased, and the destruction of the dextrose was complete, the mixture was filtered, and the liquid evaporated at 90° under diminished pressure. The syrup thus obtained was only slightly dextrorotatory. Attempts were made to crystallise the sugar from absolute alcohol and ethyl acetate, but only amorphous flakes were formed, whilst its concentrated aqueous solution did not crystallise after being kept for a long time. A portion of the syrup was therefore employed for the preparation of an osazone, which, when crystallised from dilute alcohol, separated in clusters of yellow needles, melting at 157—158°:

0·1050 gave 0·2396 CO_2 and 0·0600 H_2O. C = 62·2; H = 6·3.

$C_{17}H_{20}O_3N_4$ requires C = 62·2; H = 6·1 per cent.

The result of this analysis renders it evident that the osazone is derived from a pentose, and, furthermore, an examination of the products of hydrolysis of the disaccharide, as described below, indicated that this pentose possesses an optical rotation equal to that of xylose. When the osazone melting at 157—158° was mixed with a specimen of xylosazone of known purity, no depression of the melting point was observed. There can consequently be little doubt that the pentose formed in the above-described hydrolysis is xylose, although the isolation of the latter in a crystalline state was not effected.

The formation of dextrose and xylose by the acid hydrolysis of the disaccharide is therefore to be represented by the following equation:

$$C_{11}H_{20}O_{10} + H_2O = C_6H_{12}O_6 + C_5H_{10}O_5.$$

Inversion of Glucoxylose.—As already stated, aqueous solutions of glucoxylose are lævorotatory, but on heating with mineral acids they become dextrorotatory, owing to the formation of dextrose and xylose. This change, being analogous to that produced when sucrose is inverted, may be termed the inversion of glucoxylose, although the optical rotations of the two primary sugars and their products of inversion are in opposite directions.

Preliminary experiments showed that the change of rotation is rapidly completed by heating the glucoxylose for ten minutes at a temperature of 80° with mineral acids of 5 per cent. strength. The following experiments were according conducted:

(1) 1·2780 Grams* of dibenzoylglucoxylose (= 0·7668 gram of glucoxylose) were converted into the disaccharide, as previously described, and the latter was dissolved in 100 c.c. of water. This solution gave $\alpha_D - 0°33'$ in a 2-dcm. tube. It was then inverted by the addition of 10 c.c. of concentrated hydrochloric acid and heating for ten minutes, when the rotation in a 2-dcm. tube became $\alpha_D + 0°29'$.

(2) 1·1050 Grams* of dibenzoylglucoxylose (= 0·6630 gram of glucoxylose) were converted into the disaccharide, and the latter was dissolved in 100 c.c. of water. This solution gave $\alpha_D - 0°29'$ in a 2-dcm. tube, and after inversion the rotation was $\alpha_D + 0°26'$.

With the assumption that the hydrolytic products of the disaccharide are dextrose ($[\alpha]_D + 52·5°$) and xylose ($[\alpha]_D + 18·1°$), it is possible to calculate the rotation after inversion by means of the equation:

$$\alpha_D = lp\frac{(180\ A + 150\ B)}{312 \times 100},$$

where l = the length in decimetres of the tube in which the rotation is observed, p the weight of glucoxylose (M.W. = 312) represented by 100 c.c. of the final liquid after inversion, and A and B the specific rotations of dextrose (M.W. = 180) and xylose (M.W. = 150) respectively. Applying this equation to the experiments noted above, the calculated rotations are: (1) $\alpha_D + 0°33'$, and (2) $\alpha_D + 0°28'$, whilst the rotations found by experiment were $\alpha_D + 0°29'$ and $\alpha_D + 0°26'$ respectively.

It is thus seen that the rotation of the hydrolytic products of glucoxylose is in agreement, within the limits of experimental error, with the calculated results.

Since pure glucoxylose has no reducing action on Fehling's solution, it is to be concluded that it does not contain a carbonyl group. It must, therefore, be represented by the following constitutional formula:

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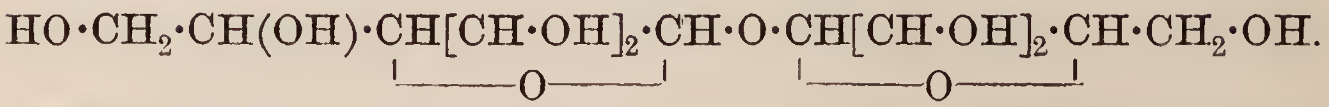
* Anhydrous substance.



$$\mathrm{HO\cdot CH_2\cdot CH(OH)\cdot \underbrace{CH[CH\cdot OH]_2\cdot CH}_{O}\cdot O\cdot \underbrace{CH[CH\cdot OH]_2\cdot CH}_{O}\cdot CH_2\cdot OH.}$$

The position of the benzoyl groups in dibenzoylglucoxylose has not as yet been ascertained.

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